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Demonstration of primary and asymptomatic DNAaemia in participants challenged with *Salmonella* Typhi (Quailes strain) during the development of a human model of typhoid infection

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Background: Typhoid infection remains a major cause of global morbidity. Effective vaccination programmes and new diagnostic tests are urgently needed but are hindered by incomplete understanding of *S.*Typhi pathogenesis, in part due to insufficiently sensitive methods for detecting bacteria in the peripheral circulation of those encountering infection. Here, new insights into *S.*Typhi pathogenesis gained using a culture-PCR methodology during a human typhoid challenge model are described.

Methods: 40 healthy adult participants were challenged with *S.*Typhi (Quailes strain) at doses of 1-5 × 103 or 1-5 × 104 colony-forming units. During the 2-weeks after challenge, participants were reviewed daily with clinical data and specimen collection, including blood drawn for 'routine' microbiological culture and a novel culture-PCR assay. For this, 5 mL venous whole-blood was collected into heparin prior to 5-hour culture in 5% ox-bile tryptone soya broth. Centrifugation was performed to collect the blood pellet/bacteria and DNA was extracted using a commercial bloodspin kit. PCR using primers to amplify the *fliC-d* gene was performed.

Results: Bacterial DNA was detected in the peripheral circulation in 57/684 (8.3%) culture-PCR and 53/674 (7.9%) routine blood culture samples. Positive culture-PCR results were detected from 12 hours after oral challenge; 10/40 participants had positive culture-PCR results (but negative routine cultures) within 5 days of challenge. Seven of these participants went on to develop typhoid infection during the 2-week challenge period (typhoid diagnosis defined by development of bacteraemia or persistent fever >38 °C for >12-hours). DNA was detected in the peripheral circulation of 5/40 participants who were not diagnosed with typhoid infection during the challenge period. Several of these participants had mild symptoms or elevation of inflammatory markers (including C-reactive protein) only.

Conclusion: These data suggest that a culture-PCR methodology targeting the *fliC-d* gene may be used to detect DNA in peripheral blood of those challenged with*S.*Typhi. Aside from unique confirmation that the mechanism of typhoid infection includes primary dissemination of bacteria in the peripheral circulation, we also demonstrate that asymptomatic infection/circulation of bacteria maybe more common than previously anticipated. Sensitive detection of *S.*Typhi DNA in peripheral blood samples may represent a useful additional endpoint in the evaluation of typhoid vaccines.

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First report of a natural reservoir of emetic *Bacillus weihenstephanensis*

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Background: *B. weihenstephanensis* has been described as psychrotolerant *B. cereus* strain producing emetic and diarrhoeal type enterotoxins. This new species grow at 4-7oC but not at 43oC and this new species can be identified by 16SrDNA and *cspA* targeted PCRs. This study was designed to explore earthworms as a possible natural habitat of this bacteria.

Methods: Two litter-dwelling earthworm species commonly used for vermicomposting, e.g. *Eisenia foetida* (exotic) and *Perionyx excavatus* and two earthworms commonly found in Indian soils, *Metaphire posthuma* and *Lampito mauritii* were selected for the study. Microorganisms from the gut of these earthworms were isolated and identified by standard techniques. This was followed by isolation of their genomic DNA, amplifications by PCR and bacterial typing-comparison with database. Sequence data was aligned and analyzed for finding the closest homologs for the microbe from National Center for Biotechnology Information (NCBI GenBank) and The Ribosomal Database Project (RDP database).

Results: Based on nucleotides homology and phylogenetic analysis the microbe obtained from the gut of *P. excavatus* was detected repeatedly to be *Bacillus weihenstephanensis.* (GenBank Accession Number: DQ345791).

Conclusion: This study described the gut of the earthworm *P. excavatus* as the natural habitat of *Bacillus weihenstephanensis.*

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Bacteriological profile and susceptibility pattern of neonatal blood stream infections

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Background: Neonatal blood stream infection is a major cause of neonatal mortality and morbidity. Due to the low sensitivity and reporting delay of blood cultures, presumptive treatment usually starts with a broad spectrum antimicrobial agents. It is therefore necessary to periodically review and analyze the bacteriological profiles and susceptibility pattern of common isolates to help the local physicians in designing management strategies.

Methods: One thousand three hundred fifty two blood culture requests from neonates were analyzed for a period of one year in a referral tertiary hospital in Sharjah, United Arab Emirates. Bacteriological profiles were obtained from BacT/ALERT® 3D system. Identification and susceptibilities studies were completed by MicroScan Walk-Away®. Risk factors for sepsis in the neonates were recorded.

Results: Blood culture was positive in 61 (4.5%) of cases. Gram-negative bacteremia was encountered in 61% of the culture-positive cases. *Escherichia coli* and *Klebsiella* species were the predominant pathogens amongst gram-negative organisms. Most gram-negative organisms were sensitive to carbapenems including imipenem and meropenem, tigecycline, and ceftriaxone. The most common gram-positive organism isolated was *Staphylococcus aureus* (26%). All gram-positive were sensitive to vancomycin and tigecycline. The most important risk factor of bacteremia in our study population was preterm birth (53%).

Table 1: Distribution of bacterial isolates

Organism isolated	**No. of cases**	
Gram positive	*Staphylococcus aureus*	16
	Coagulase negative staphylococci	01
	Enterococcus species	03
	Streptococcus aglacitae	03
	Streptococcus pyogenes	01
Gram negative	*Escherichia coli*	16
	Enterobacter species	02
	Klebsiella species	07
	Salmonella species	03
	Acinetobacter species	03
	Stenotrophomonas maltophilia	01
	Pseudomonas species	04
	Chryseobacterium meningosepticum	01
Total isolates		61

Table 2: Sensitivity pattern of the Gram-positive isolates

Antibiotics	*Staphylococcus aureus* (n = 16)	other Gram-positive organisms (n = 8)
Amoxycillin	7 (40)	6(75)
Penicillin	0(0)	6 (75)
Tigecycline	16 (100)	8 (100)
Vancomycin	16 (100)	8 (100)
Co-trimoxazole	13(82)	6(75)
Ciprofloxacin	12 (75)	-

N.B. (-) means that it has not been done; Figures in parentheses are in percentage

Table 3: Sensitivity pattern of the Gram-negative isolates (percentage sensitive)

Antibiotics	*E.coli* (n = 16)	*Entero* (n = 2)	*Klebsiella* (n = 7)	Salmonella (n = 3)	Acineto (n = 3)	Pseudo (n = 4)
Tigecycline	16(100)	2(100)	7(100)	3(100)	0 (0)	-
Ceftriaxone	13(81)	2(100)	4(58)	3100)	0(0)	3(75)
Cefotaxime	11(69)	1(50)	4(58)	3(100)	0(0)	0(0)
Imipenem	16(100)	2(100)	7(100)	3(100)	3(100)	4(100)
Meropenem	16(100)	2(100)	7(100)	3(100)	3(100)	4(100)
Gentamicin	08(50)	2(100)	3(42)	-	0(0)	4(100)
Amikacin	10(62)	2(100)	3(42)	-	1(33)	4(100)
Ciprofloxacin	13(81)	1(50)	5(71)	3 (100)	0(0)	3(75)

N.B. (-) means that it has not been done.**Conclusion:** From the susceptibility pattern it appears, tigecycline, imipenam and third generation cephalosporins have replaced amoxicillin-clavulanic acid, co-trimoxazole, and gentamicin plus beta-lactam as suitable agents for empiric therapy of neonatal blood stream infections in our setup. However, due to limitations in our sample size, we will continue to perform periodic antimicrobial susceptibility surveillance to create a dynamic database useful to the local physician in treating neonatal blood stream infections.

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Metabolomics based biomarker discovery for infectious diseases, the case of melioidosis

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Background: The current diagnostic arsenal for infectious diseases is predominantly based on pathogen-detection, but is defied by many lethal pathogens that can cause disease while being hidden in the body or simply undetectable. Metabolomics has great potential to offer new diagnostic solutions as it provides the possibility to identify new disease biomarkers in the form of pathogen-responsive metabolites in body fluids that can be translated to bedside diagnostics. In addition, metabolite biomarkers for disease could further point out which human metabolic processes are disrupted by the infecting pathogen and as such provide a better understanding of the underlying disease. We here present a study that evaluates metabolomics as an applied research strategy for improving infectious disease control. Our work focuses on the bacterial disease melioidosis for which new diagnostic tools are needed to reduce its associated morbidity and mortality in Australia and Southeast Asia.

Methods: Blood samples for metabolic profiling were collected in the melioidosis hyperendemic Khon Kaen Province in Thailand from (i) non-infected controls, (ii) patients with bacteremia due to other bacteria, and (iii) bacteremic melioidosis patients. Blood plasma was analyzed by gas-chromatography mass-spectrometry (GC-MS) to characterize the polar metabolites, and liquid-chromatography mass-spectrometry (LC-MS) to characterize the non-polar metabolites.

Results: The current results suggest that plasma metabolic profiles can robustly differentiate bacteremic melioidosis patients from patients suffering from other bacterial blood infections and healthy controls from the same endemic region. Various compounds were found to have a characteristic profile in melioidosis patients and represent new candidate diagnostic biomarkers for this lethal disease.

Conclusion: We will present these findings in the context of diagnostic biomarker discovery and highlight the power of metabolic profiling to reveal pathogenetic differences amongst melioidosis patients. The potential strengths offered by metabolomics for infectious disease research will be discussed.

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